Trans-fatty acid intake in relation to serum lipid concentrations in adult men[1-3]

Rebecca Troisi, Walter C Willett, and Scott T Weiss

ABSTRACT The relation of *trans*-fatty acid intake to fasting serum lipid concentrations was evaluated in a cross-sectional study of 748 men aged 43–85 y. Multiple-linear-regression analysis was used to adjust for age, body mass index, waist-to-hip circumference ratio, smoking status, physical activity, alcohol intake, total energy, dietary cholesterol and linoleic acid, and previous serum cholesterol concentration. *Trans*-fatty acid intake was directly related to total serum ($r = 0.07$, $P = 0.04$) and low-density-lipoprotein cholesterol (LDL) ($r = 0.09$, $P = 0.01$), and inversely related to high-density-lipoprotein (HDL) cholesterol ($r = 0.08$, $P = 0.03$). *Trans*-fatty acid intake was positively associated with the ratios of total to HDL cholesterol ($r = 0.11$, $P = 0.002$) and LDL to HDL cholesterol ($r = 0.12$, $P = 0.001$). The estimated ratios of total to HDL cholesterol were 4.4 and 4.9 for persons at the 10th (2.1 g/d) and 90th (4.9 g/d) percentiles of *trans*-fatty acid intake, respectively. On the basis of results from other studies, these ratios would correspond to a 27% increase in risk of myocardial infarction. *Am J Clin Nutr* 1992;56:1019–24.

KEY WORDS *Trans*-fatty acids, serum lipids, diet

Introduction

The effects of dietary fats on blood lipid concentrations have been examined in numerous studies. Certain saturated fatty acids, especially palmitic acid, and dietary cholesterol raise serum low-density-lipoprotein-cholesterol (LDL-C) concentrations, and polyunsaturated and monounsaturated fatty acids appear to be desirable substitutes (1). Less is known about the effects on serum lipids of *trans*-fatty acids, a source of unsaturated fatty acid in the American diet that has greatly increased over this century.

Trans-fatty acids are produced by partial hydrogenation of unsaturated fatty acids, a process used to saturate double bonds in which some double bonds are converted from their normal *cis* configuration to the *trans* isomer (2). Partial hydrogenation is used to convert liquid vegetable oils to solid fats such as margarine and shortening, which are often consumed with the expectation of reducing the risk of coronary heart disease. The major sources of *trans*-fatty acids in the US diet are margarine and spreads, food-service fats and oils, and meat and dairy products (3).

Mensink and Katan (4) recently reported the results of an experimental trial designed to evaluate the effect of *trans* isomers of oleic acid on serum lipid concentrations. *Trans*-fatty acid intake was associated with an adverse lipoprotein profile: higher intake increased LDL-C concentrations and decreased high-density-lipoprotein-cholesterol (HDL-C) concentrations. Increases in serum total cholesterol concentrations with increased intake of *trans*-fatty acids have been reported in some (5, 6) but not all (7–11) feeding experiments in humans, but most of these earlier studies did not specifically measure LDL-C and HDL-C. The study of Mensink and Katan has been criticized for using *trans* isomers obtained by a process not typical of hydrogenation of margarines and shortenings in the United States (12). Their study was further criticized for using higher amounts of *trans*-fatty acids than are characteristic of the US diet (12). In this study we address these issues of generalizability by assessing the relationship of *trans*-fatty acid intake to serum lipid concentrations in a large population-based cohort of US men.

Subjects and methods

The Normative Aging Study (NAS) is an ongoing longitudinal, multidisciplinary study established by the Veterans' Administration in 1961. Details of the study protocol have been presented elsewhere (13). Male volunteers were screened for a variety of medical conditions, including hypertension, cancer, and diabetes, to identify an initially healthy population. Body weight and hyperlipidemia were not screening criteria. Subjects have received biomedical and anthropometric examinations every 3–5 y since 1961.

The protocol for this substudy was approved by the Human Studies Subcommittee of the Research and Development Committee, Department of Veterans' Affairs, Veterans' Administration outpatient clinic; written informed consent was obtained from all subjects.

Subjects

For this analysis we used information collected from examinations conducted between February 1987 and April 1990. Data on dietary intake, serum lipids, and other covariates were avail-

[1] From the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; the Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston; and the Pulmonary Division, Beth Israel Hospital, Boston.

[2] Supported by grant HL37871 from the Division of the Heart, Lung, and Blood Institute, and Clinical Epidemiology of Lung, and Heart Disease Research Service award HL07427.

[3] Address reprint requests to ST Weiss, Channing Laboratory, 180 Longwood Avenue, Boston, MA 02115.

Received December 2, 1991.

Accepted for publication June 24, 1992.

able for 985 men out of a total of 1133 examined during this period. Two-hundred thirty-seven men were excluded from the analysis because they were taking medication that could affect blood lipids: hypoglycemic medication (n = 28), diuretics (n = 45), and hypolipemic medication (n = 164); 748 men met the inclusion criteria.

Dietary intake and physical activity

Dietary intake was assessed by a semiquantitative food frequency questionnaire (14, 15) consisting of 116 food items with serving sizes. Type of fat ordinarily used for baking and for frying food, brand and type of cooking oil, and type of margarine (stick or tub) are also asked on the questionnaire. Fatty acid intake was computed by multiplying the frequency of consumption of each food item by the fatty acid composition for the portion size specified, and summing across all foods (16). Subjects were mailed the questionnaire and asked to complete it before their visit to the study. Each questionnaire was independently coded by three researchers. A dietitian who was involved in the development of the questionnaire was consulted when coding was found to be inconsistent among the three coders. Calculations included the specific information on the fat content of types of margarines, and the usual types of fat or oil each individual used for frying, cooking, and baking. The nutrient-composition data are based primarily on US Department of Agriculture publications (17) and supplemented by other published sources and personal communications from laboratories and food manufacturers (18–22). **Table 1** gives the values used in the calculation of *trans*-fatty acids from some of the major food sources. Values for total energy intake, dietary intake of alcohol, cholesterol, and saturated, polyunsaturated, monounsaturated, *trans*, stearic, palmitic, linoleic, and oleic fatty acids are derived from the questionnaire. Energy-adjusted nutrient intakes for cholesterol and fats were computed by regression analysis (23). This was accomplished by computing residuals from regression models with total energy as the independent variable and absolute nutrient intake as the dependent variable. The residuals were then added to the expected nutrient value at the mean energy intake of the study sample to provide an intuitive value for nutrient intake. The ratio of polyunsaturated to saturated fatty acid (P:S) intake was calculated.

The questionnaire we used also provides information on physical activity. Based on the scale of Paffenbarger et al (24), questions on the number of flights of stairs climbed per day, walking pace, and the frequency of various physical activities were used to derive total energy (in J) expended for exercise per week. The distribution of energy used per week was divided into quintiles.

Serum lipid measurements

Serum lipids were measured at the Veterans' Administration Outpatient Clinic in Boston from February 1987 to July 1989, and at the Veterans' Administration Hospital in Jamaica Plains, MA, from August 1989 to April 1990. One blood sample for lipid determinations was drawn after a minimum of an 8-h fast and abstinence from smoking. One 15-mL evacuated tube was used to measure total cholesterol, triglyceride concentration, and HDL-C. Cholesterol was measured by using an enzymatic assay (SCLAVO Diagnostics, SCLAVO Inc, Wayne, NJ). Triglyceride concentrations were determined by using the TGL triglyceride test (DuPont Company, Biomedical Products Department, Wilmington, DE). HDL-C was determined by using dextran sulfate and magnesium to precipitate lipoproteins except HDL-C, which remains in the supernatant. The HDL-C fraction was then quantified by using the Abbott Bichromatic Analyzer 100. The intraclass coefficient of variation (for laboratory variance only) was 1.5% for total serum cholesterol and 5.3% for HDL-C. LDL-C was calculated by using the following equation:

$$\text{Total cholesterol} - \text{HDL-C} - (\text{serum triglyceride}/5)$$

The ratios of total serum cholesterol to HDL-C, and LDL-C to HDL-C were calculated.

Subjects in the NAS are mailed laboratory results from the cholesterol determinations a few weeks after their examinations. In previous cycles of data collection, subjects with serum total cholesterol concentrations $\geq$ 6.47 mmol/L were informed that they had a high total serum cholesterol value and were advised to contact their private physicians. For the present analyses laboratory values for total serum cholesterol from the preceding NAS examination [(mean $\pm$ SD) from previous to present examination, 3.6 $\pm$ 1.3 y] were used to divide subjects into two groups: *1*) subjects previously informed that their total serum cholesterol concentration was high, and *2*) subjects not informed that their cholesterol concentration was high. The classification was performed because we hypothesized that subjects' awareness of a high total serum cholesterol concentration might modify their intake of fat or affect their reporting of fat types on the dietary questionnaire. An indicator variable for the two groups was created for use in the regression analyses. Anthropometric measurements and information on smoking were described elsewhere (25).

Statistical analyses

Pearson product-moment and partial correlations were used to relate *trans*-fatty acid intake to serum lipid concentrations by using logarithm transformed variables to improve normality.

TABLE 1
Trans-fatty acid values used to calculate amount per serving of selected foods and percent contribution of the food to total *trans*-fatty acid intake

Food	*Trans*-fatty acids	Contribution of food
	g/serving	%
Margarine (5 g)*	—	12.61
Stick	0.8	—
Tub	0.6	—
Diet	0.3	—
Beef (124–186 g)	1.3	9.62
Fried potatoes (124 g)	2.69	7.91
Oil and vinegar dressing (15 mL)	0.8	5.66
Ready-made cookie (one)	0.6	5.27
Ready-made sweet roll, coffee cake, pastry (one)	1.6	4.77
Hamburger patty (124 g)	0.8	4.35
Mayonnaise (15 mL)	0.5	3.57
Cheese (31 g)	0.3	3.39
Beef sandwich (93 g)	0.55	2.76

* US Department of Agriculture value for margarines made with soybean oil.

TABLE 2
Current serum lipids and current dietary intake, by total cholesterol concentrations, on previous examination

	< 6.47 mmol/L (n = 373)	≥ 6.47 mmol/L (n = 375)	P
Serum cholesterol (mmol/L)			
Total	5.5 ± 0.85*	6.6 ± 0.85	
LDL	3.6 ± 0.77	4.6 ± 0.82	0.0001
HDL	1.2 ± 0.31	1.3 ± 0.32	0.08
Triglyceride	3.3 ± 1.6	3.9 ± 1.9	0.0001
Total:HDL	4.7 ± 1.3	5.5 ± 1.5	0.0001
LDL:HDL	3.1 ± 1.0	3.8 ± 1.2	0.0001
Dietary intake			
Total energy (kJ)	8382 ± 2684	8244 ± 2433	0.46
Cholesterol (g)	275 ± 123	243 ± 103	0.0001
Saturated fatty acid (g)	24.1 ± 10.0	22.5 ± 9.7	0.03
Polyunsaturated fatty acid (g)	12.4 ± 5.4	12.3 ± 5.2	0.79
Monounsaturated fatty acid (g)	25.2 ± 10.2	24.2 ± 10.1	0.17
P:S†	0.54 ± 0.19	0.59 ± 0.22	0.003
Trans-fatty acids (g)	3.7 ± 1.8	3.6 ± 1.8	0.50

* $\bar{x}$ ± SD.
† Ratio of polyunsaturated to saturated fatty acid intake.

Multiple-linear-regression analysis was used to adjust for the influences of age, body mass index (BMI), waist-to-hip ratio, current and former smoking, alcohol intake, physical activity, total energy, and dietary cholesterol intake. Models were run with and without the indicator variable for serum cholesterol status on last examination, and intake of other fatty acids. Analyses were performed by using the Statistical Analysis System *AOS/VS* version 5.18 and *UNIX* version 6.03 (26).

Results

Mean (±SD) age for the study sample was 62 ± 7.83 y (range 43–85 y) and mean (±SD) BMI was 26.3 ± 3.32 (in kg/m^2) (range 15.6–47.1). Age and BMI did not significantly differ between the groups with and without elevated serum cholesterol on the earlier examination. Men with total serum cholesterol values ≥ 6.47 mmol/L on their previous examination had significantly greater total serum cholesterol, LDL-C, and triglyceride values on their most current examination (**Table 2**). As anticipated, differences in dietary intake between the two groups were also observed. Total energy intake did not differ between groups but subjects with higher cholesterol values on their previous examination reported eating significantly less dietary cholesterol and saturated fatty acids, and had a higher P:S at their current examination. Intakes of polyunsaturated, monounsaturated, and *trans*-fatty acids did not significantly differ by previous cholesterol status. Mean energy-adjusted intake of *trans*-fatty acids for the entire sample was 3.4 ± 1.2 g/d ($\bar{x}$ ± SD). *Trans*-fatty acid intake unadjusted for total energy constituted 5.5% of total fat and 1.6% of total energy.

As indicated in **Table 3,** energy-adjusted *trans*-fatty acid intake was positively correlated with LDL-C and inversely correlated with HDL-C. Intake was positively associated with the ratio of serum total cholesterol to HDL-C and the ratio of LDL-C to HDL-C. Adjustment for energy intake, age, BMI, waist-to-hip ratio, current and former smoking, physical activity, and alcohol intake attenuated the strength of these correlations, primarily because of the strong positive association of alcohol intake and HDL-C (r = 0.29) and inverse correlation of alcohol intake with energy-adjusted *trans*-fatty acid intake (r = −0.24). Further adjustment for previous cholesterol concentration (by using the indicator variable) increased slightly the correlation between *trans*-fatty acid intake and LDL-C; the other correlations were unchanged. Spearman rank correlations gave results similar to those attained with Pearson product-moment correlations.

Multiple-linear-regression analysis was used to determine the quantitative relationships between *trans*-fatty acid intake and serum lipids after adjustment for factors known to affect lipid concentrations. The basic model included *trans*-fatty acid intake, total energy, age, BMI, waist-to-hip ratio, smoking status, alcohol intake, physical activity, and dietary cholesterol intake with serum lipid concentration as the outcome variable. A second set of regression models included energy-adjusted linoleic acid because linoleic acid was strongly correlated with *trans*-fatty acid intake (r = 0.78, P = 0.0001) and also lowers LDL-C (1). A third set of regression models included an indicator variable to represent previous serum cholesterol status. **Table 4** represents energy-adjusted *trans*-fatty acid intake as a predictor of serum lipid concentrations. In the model with serum total cholesterol as the outcome, the slope for *trans*-fatty acids was positive (P

TABLE 3
Simple and partial correlations (P values) between *trans*-fatty acid intake and serum lipid concentrations

	Simple	Energy-adjusted	Multivariate-adjusted*	Multivariate-adjusted†
Cholesterol				
Total	0.01 (0.89)	0.03 (0.41)	0.05 (0.18)	0.07 (0.06)
LDL	0.03 (0.40)	0.07 (0.05)	0.07 (0.06)	0.09 (0.01)
HDL	−0.08 (0.03)	−0.18 (0.0001)	−0.03 (0.35)	−0.03 (0.36)
Total:HDL	0.08 (0.04)	0.17 (0.0001)	0.07 (0.07)	0.07 (0.05)
LDL:HDL	0.08 (0.03)	0.18 (0.0001)	0.08 (0.04)	0.09 (0.02)
Triglyceride‡	0.00 (0.93)	0.06 (0.12)	−0.01 (0.89)	−0.00 (0.94)

* Adjusted for energy intake, age, BMI, waist-to-hip ratio, smoking status, physical activity, and alcohol intake.
† Adjusted for energy intake, age, BMI, waist-to-hip ratio, smoking status, physical activity, alcohol intake, and previous cholesterol concentration (≥ or < 6.47 mmol/L on last examination).
‡ Triglyceride is logarithm transformed.

TABLE 4
Regression coefficients and predicted serum lipid concentrations in relation to energy-adjusted *trans*-fatty acid intake*

	β (SE)†	P	Percentile of *trans* isomer intake‡ 10%	90%
Total cholesterol				
trans-fatty acids	0.25 (0.11)	0.09	6.0	6.2
With linoleic acid	0.22 (0.13)	0.09	6.0	6.2
With cholesterol status	0.22 (0.11)	0.04	5.4§	5.6
LDL (mmol/L)				
trans-fatty acids	0.23 (0.10)	0.02	4.0	4.2
With linoleic acid	0.26 (0.12)	0.03	4.0	4.2
With cholesterol status	0.26 (0.10)	0.01	3.5	3.7
HDL (mmol/L)				
trans-fatty acids	−0.04 (0.03)	0.17	1.3	1.2
With linoleic acid	−0.08 (0.03)	0.03	1.3	1.2
With cholesterol status	−0.08 (0.03)	0.03	1.3	1.2
Total cholesterol:HDL cholesterol				
trans-fatty acids	0.34 (0.14)	0.02	4.9	5.2
With linoleic acid	0.50 (0.17)	0.004	4.8	5.3
With cholesterol status	0.50 (0.16)	0.002	4.4	4.9
LDL:HDL				
trans-fatty acids	0.31 (0.12)	0.008	3.2	
With linoleic acid	0.42 (0.14)	0.002	3.4	3.8
With cholesterol status	0.43 (0.13)	0.001	2.9	3.3

* All values adjusted for energy intake, age, BMI, waist-to-hip ratio, smoking status, alcohol intake, physical activity, and dietary cholesterol intake; values for "with linoleic acid" were also adjusted for linoleic acid intake (prediction uses 2.26 $\log_e$ g/d); values for "with cholesterol status" were also adjusted for linoleic acid intake and an indicator variable for cholesterol status on last examination (total serum cholesterol concentration ≥ or < 6.47 mmol/L).

† Regression coefficients represent the change in serum cholesterol values in mmol/L per unit increase in *trans*-fatty acid intake. Units for intake of *trans*-fatty acid are in $\log_e$ g/d.

‡ Predicted lipid values for an individual at the 10th and 90th percentiles of *trans*-fatty acid intake, assuming the mean value of age (62 y), BMI (26.31), waist-to-hip ratio (0.97), alcohol intake (16.69 g/d), energy-adjusted dietary cholesterol intake (5.47 $\log_e$ g/d) in the third level of physical activity, and nonsmoking.

§ In the regression equation used to predict lipid values at the 10th and 90th percentiles of *trans*-fatty acid intake, the value for cholesterol status is 0 (corresponding to a previous cholesterol concentration < 6.47 mmol/L).

= 0.04) when both linoleic acid and cholesterol status were added to the model. *Trans*-fatty acid intake was more strongly related to LDL-C than to total serum cholesterol. The inverse relation of *trans*-fatty acid intake to HDL-C doubled with the addition of linoleic acid to the model (β; = -0.04 mmol · L $^{-1}$ · $\log_e$g $^{-1}$ · d^{-1} of *trans*-fatty acid intake vs $\beta = -0.08$). *Trans*-fatty acid intake was positively associated with the ratios of total serum cholesterol to HDL-C, and LDL-C to HDL-C; these associations increased with the addition of linoleic acid to the model. The relation of *trans*-fatty acid intake on serum lipid measures and ratios was similar when linoleic acid was replaced by saturated, polyunsaturated, and monounsaturated fatty acids in separate regression models (**Table 5**).

Interaction variables were formed to determine whether the association between *trans*-fatty acid intake and serum lipid concentrations depended on total serum cholesterol concentration (≥ or < 6.47 mmol/L) on the previous examination. The results of this analysis suggested that the relation between *trans*-fatty acid intake and both total serum cholesterol and LDL-C concentrations was stronger in the men who had total serum cholesterol concentrations ≥ 6.47 mmol/L 3–5 y earlier (tests for interaction, $P = 0.05$ and $P = 0.07$, respectively). Results were similar when stratified by cholesterol status.

The models in Table 4 were used to estimate serum lipid concentrations for persons with energy-adjusted intake of *trans*-fatty acids at the 10th and 90th percentiles (2.1 and 4.9 g/d, or 1.0% and 2.4% of total energy, respectively). The mean values for age, BMI, waist-to-hip ratio, alcohol intake, and dietary cholesterol and linoleic acid intakes were used for a never-smoker in the third quintile of physical activity with a total serum cholesterol value < 6.47 mmol/L on the last examination. Predicted mean LDL-C concentration corresponding to the 10th percentile of *trans*-fatty acid intake was 3.52 mmol/L and increased to 3.75 mmol/L for the 90th percentile. Similarly, for men at the 10th and 90th percentiles of *trans*-fatty acid intake, predicted values were, respectively, 1.28 and 1.21 mmol/L for HDL-C, 4.4 and 4.9 for the ratio of total serum cholesterol to HDL-C, and 2.9 and 3.3 for the ratio of LDL-C to HDL-C.

TABLE 5
Regression coefficients relating serum lipids to energy-adjusted *trans*-fatty acid intake*

	β† (SE)	P
Total serum cholesterol (mmol/L)		
trans-fatty acids		
With saturated fatty acid	0.21 (0.12)	0.08
With polyunsaturated fatty acid	0.20 (0.12)	0.10
With monounsaturated fatty acid	0.18 (0.14)	0.20
LDL (mmol/L)		
trans-fatty acids		
With saturated fatty acid	0.27 (0.11)	0.01
With polyunsaturated fatty acid	0.24 (0.11)	0.03
With monounsaturated fatty acid	0.26 (0.13)	0.04
HDL (mmol/L)		
trans-fatty acids		
With saturated fatty acid	−0.07 (0.03)	0.04
With polyunsaturated fatty acid	−0.07 (0.04)	0.04
With monounsaturated fatty acid	−0.10 (0.04)	0.01
Total cholesterol:HDL cholesterol		
trans-fatty acids		
With saturated fatty acid	0.48 (0.16)	0.003
With polyunsaturated fatty acid	0.47 (0.17)	0.005
With monounsaturated fatty acid	0.55 (0.19)	0.004
LDL cholesterol:HDL cholesterol		
trans-fatty acids		
With saturated fatty acid	0.42 (0.13)	0.001
With polyunsaturated fatty acid	0.40 (0.13)	0.003
With monounsaturated fatty acid	0.46 (0.15)	0.003

* Adjusted for energy intake, age, BMI, waist-to-hip ratio, smoking status, alcohol intake, physical activity, and dietary cholesterol intake with saturated, polyunsaturated, and monounsaturated fatty acids added separately to the models.

† Change in serum cholesterol values in mmol/L per unit increase in *trans*-fatty acid intake. Units for intake of *trans*-fatty acids are in $\log_e$ g/d.

Multiple-linear-regression analyses were also performed replacing *trans*-fatty acids with margarine intake (**Table 6**), a major source of *trans* isomers, from the dietary questionnaire. These analyses yielded results similar to those obtained for *trans*-fatty acids (for the ratio of total cholesterol to HDL-C, $\beta = 0.034 \pm 0.01 \cdot g\ margarine^{-1} \cdot d^{-1}$, $P = 0.001$).

TABLE 6
Regression coefficients relating serum lipids to margarine intake*

	β† (SE)	P
Total cholesterol	0.016 (0.008)	0.04
LDL	0.018 (0.006)	0.01
HDL	−0.006 (0.002)	0.02
Total cholesterol:HDL	0.034 (0.01)	0.001
LDL:HDL	0.028 (0.008)	0.001

* Adjusted for age, BMI, waist-to-hip ratio, smoking status, alcohol intake, physical activity, total energy intake, energy-adjusted dietary cholesterol intake, and energy-adjusted linoleic acid intake.

† Difference in serum cholesterol values (mmol/L) per g/d intake of margarine.

Discussion

In this cross-sectional study, intake of *trans*-fatty acids was associated with an adverse lipoprotein profile; *trans*-fatty acids were positively related to LDL-C and inversely related to HDL-C. Intake of *trans*-fatty acids also was positively related to the ratios of total serum cholesterol to HDL-C, and LDL-C to HDL-C. Similar associations were seen with intake of margarine, a major source of dietary *trans*-fatty acids.

Earlier experimental trials evaluated the effect of partially hydrogenated oil on total serum cholesterol but not on cholesterol subfractions (5–7, 9–11). Negative findings in several of these trials might be explained by the opposite effect on LDL-C and HDL-C, and are consistent with the relatively weak effect of *trans*-fatty acids on total serum cholesterol demonstrated in this study. The stronger effects of *trans*-fatty acids on LDL-C and HDL-C reported by Mensink and Katan (4) were substantiated in the present study. In their study 10% of total energy intake was replaced by *trans*-fatty acids, resulting in an increase in total serum cholesterol and LDL-C and a decrease in HDL-C. The increase in LDL-C noted by these investigators was not as substantial as the decrease in HDL-C. The authors concluded that *trans*-fatty acids were associated with a lipoprotein profile at least as adverse as that resulting from saturated fatty acid intake.

The finding that the effect of *trans*-fatty acids on LDL-C was greater in subjects with a history of a high total serum cholesterol concentration (≥ 6.47 mmol/L) may have important implications. Although not demonstrated in the present data, individuals with high serum cholesterol may be more likely to substitute products containing *trans*-fatty acids for saturated fatty acid and cholesterol. To the extent that the effect of *trans*-fatty acids on serum may be greater in these individuals, they would represent a subgroup who would experience increased susceptibility to the effect of *trans*-fatty acids and would be at greater increased risk of coronary heart disease.

Our study adds to the finding of Mensink and Katan by demonstrating an association between intake of *trans*-fatty acids and lipid concentrations in a free-living US population with intake of *trans*-fatty acids more similar to those of the general US population. It has been estimated that 3–4% of total energy intake in the American diet consists of *trans*-fatty acids (27, 28). Estimated *trans*-fatty acid intake constituted a mean of 1.6% of total energy intake (ranging from 0.3% to 3.8%) in this sample compared with 10% in the study of Mensink and Katan (4).

Although intake of *trans*-fatty acids may have been slightly underestimated by our questionnaire, intake as a percent of fatty acids estimated by the same questionnaire corresponded closely with percent of fatty acids in subcutaneous adipose tissue samples from 115 women ($\bar{x} \pm$ SD: 5.8 ± 1.7% for intake, 4.4 ± 1.1% for adipose tissue, $r = 0.51$) (29). The results were similar in a study of 118 Boston area men (30) whose fat aspirates were collected during the period that the food frequency questionnaire was administered in the present study (4.7 ± 1.5% for intake, 4.2 ± 0.93% for adipose tissue). The mean value for *trans*-fatty acids in our data was low but within published ranges based on production figures (3). Enig et al (31) calculated a wide range of *trans*-fatty acid values (2.4–20.6 $g \cdot person^{-1} \cdot d^{-1}$) corresponding to a total fat intake of 40–258 $g \cdot person^{-1} \cdot d^{-1}$. The average total fat intake for participants in this study was ≈60 g/d, which would coincide with the lowest values of their range. It is also possible that study participants consume less processed foods (a major source of *trans*-fatty acids) than the general public, given their heightened health consciousness.

The biological mechanism whereby *trans*-fatty acids raise serum LDL-C and lower HDL-C are unknown. One possibility is suggested by studies in rats (32, 33) indicating a reduction in lecithin:cholesterol acyltransferase (LCAT) with increased ingestion of *trans*-fatty acids. The effects of LCAT deficiency—decreased ability of HDL-C to absorb cholesterol, resulting in reduced cholesterol transport to the liver (34)—may be consistent with decreased HDL-C in serum. Other studies in rats have demonstrated that some *trans*-fatty acids inhibit desaturation and elongation of polyunsaturated fatty acids (35).

The limitations of this study relate to its cross-sectional nature and the health consciousness of its subjects. Subjects informed that their cholesterol concentrations are high are likely to change their diet. Specifically, subjects may increase their use of margarine and margarine-containing products, resulting in a spurious relationship between increased intake of *trans*-fatty acids and serum cholesterol concentrations. We found, however, that intake of *trans*-fatty acids was not significantly different between subjects who were told 3–5 y earlier that they had high serum cholesterol and those who were not, suggesting that prior knowledge of serum cholesterol concentrations did not influence intake of *trans*-fatty acids. However, differential recall of dietary intake also might result if subjects who were informed that their cholesterol was high reported more favorable diets with low intakes of saturated fatty acid and cholesterol. Although it was not possible to directly assess the extent to which this potential recall bias affected reporting of fat intake by cholesterol group, an attempt was made to adjust for dietary modification and reporting by including a variable in the regression models to represent subjects' knowledge that their cholesterol concentrations were high. This adjustment had no appreciable influence on our findings. Moreover, our findings were primarily concerned with lipid subfractions rather than with total serum cholesterol.

Our analyses and those of Mensink and Katan (4) suggest a particularly strong influence of *trans*-fatty acid intake on the ratio of total cholesterol or LDL-C to HDL-C. The ratio of total serum cholesterol to HDL-C is strongly associated with coronary heart disease (36, 37). Stampfer et al (38) demonstrated a 53%

increase in risk for myocardial infarction with a one-unit change in the ratio of total cholesterol to HDL-C after adjusting for age, smoking, Quetelet's index, prior angina, hypertension, and diabetes. The predicted change in the ratio of total serum cholesterol to HDL-C from the 10th to the 90th percentile of *trans*-fatty acid intake would correspond to an increase in risk for myocardial infarction of ≈27% when this estimate is used. Because *trans*-fatty acid intake is inevitably measured imperfectly, this may represent a substantial underestimation of the expected impact.

In summary, our findings suggest that the quantity of *trans*-fatty acids in the US diet can adversely influence serum lipid concentrations. Intake of *trans*-fatty acids, primarily in the form of processed vegetable fats, was inversely related to HDL-C concentrations and positively related to LDL-C concentrations and the ratio of total cholesterol to HDL-C. The magnitude of lipid differences associated with *trans*-fatty acid intake is sufficient to have important quantitative effects on risk of myocardial infarction, the most prevalent cause of death in the United States.

References

1. Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31:1149–72.
2. Linder MC. Nutritional biochemistry and metabolism with clinical applications. New York: Elsevier Science Publishing Company, Inc, 1985.
3. Hunter JE, Applewhite TH. Reassessment of *trans* fatty acid availability in the US diet. Am J Clin Nutr 1991;54:363–9.
4. Mensink RP, Katan MB. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N Engl J Med 1990;323:439–45.
5. Vergroesen AJ. Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. Proc Nutr Soc 1972;31:323–9.
6. Anderson JT, Grande F, Keys A. Hydrogenated fats in the diet and lipids in the serum of man. J Nutr 1961;75:388–94.
7. Mattson FH, Hollenbach EJ, Kligman AM. Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. Am J Clin Nutr 1975;28:726–31.
8. Laine DC, Snodgrass CM, Dawson EA, Ener MA, Kuba K, Frantz ID. Lightly hydrogenated soy oil versus other vegetable oils as a lipid-lowering dietary constituent. Am J Clin Nutr 1982;35:683–90.
9. Grasso S, Gunning B, Imaichi K, Michaels G, Kinsell L. Effects of natural and hydrogenated fats of approximately equal dienoic acid content upon plasma lipids. Metabolism 1962;11:920–4.
10. McOsker DE, Mattson FH, Sweringen HB, Kligman AM. The influence of partially hydrogenated dietary fats on serum cholesterol levels. JAMA 1962;180:380–5.
11. Erickson A, Coots RH, Mattson FH, Kligman AM. The effect of partial hydrogenation of dietary fats, of the ratio of polyunsaturated to saturated fatty acids, and of dietary cholesterol upon plasma lipids in man. J Clin Invest 1964;43:2017–25.
12. Reeves RM. Effect of dietary *trans* fatty acids on cholesterol levels. N Engl J Med 1991;324:338–9.
13. Bell B, Rose CL, Damon A. The Normative Aging Study: an interdisciplinary and longitudinal study of health and aging. Aging Hum Dev 1972;3:5–17.
14. Sampson L. Food frequency questionnaires as a research instrument. Clin Nutr 1985;4:171–8.
15. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidermiol 1985;122:51–65.
16. Willett WC, Stampfer MJ, Underwood BA, Speizer FE, Rosner B, Hennekens CH. Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. Am J Clin Nutr 1983;38: 631–9.
17. Consumer and Food Economics Institute. Composition of food. Agriculture handbook no. 8. Washington, DC: US Government Printing Office, 1976–1989.
18. Carpenter PL, Lehman T, Mason BS, Slover HT. Lipid composition of selected vegetable oil. J Am Oil Chem Soc 1976;53:713–7.
19. Hepburn FN, Exler J, Weihrauch J. Provisional tables on the content of omega-3 fatty acids and other fat components of selected foods. J Am Diet Assoc 1986;86:788–93.
20. Slover HT, Thompson RH, Davis CA, Merola GB. Lipids in margarine and margarine-like foods. J Am Oil Chem Soc 1985;62:775–86.
21. Enig MG, Pallansch LA, Sampugna J, Keeney M. Fatty acid composition of the fat in selected food items with emphasis on trans components. J Am Oil Chem Soc 1983;60:1788–95.
22. US Department of Agriculture. Provisional table on the nutrient content of bakery foods and related items. Washington, DC: USDA, 1981.
23. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
24. Paffenbarger RS, Hyde RT, Weng AL, Hsieh CC. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986;314:605–13.
25. Troisi RJ, Heinold JW, Vokonas PS, Weiss ST. Cigarette smoking, dietary intake, and physical activity: effects on body fat distribution—the Normative Aging Study. Am J Clin Nutr 1991;53:1104–11.
26. Statistical Analysis System Institute. SAS user's guide: statistics. 5th ed. Cary, NC SAS Institute, Inc, 1985.
27. Grundy SM. *Trans* monounsaturated fatty acids and serum cholesterol levels. N Engl J Med 1990;323:480–1.
28. Hunter JE, Applewhite TH. Isomeric fatty acids in the US diet: levels and health perspectives. Am J Clin Nutr 1986;44:707–17.
29. London SJ, Sacks FM, Caesar J, Stampfer MJ, Siguel E, Willett WC. Fatty acid composition of subcutaneous adipose tissue and diet among postmenopausal US women. Am J Clin Nutr 1991;54: 340–5.
30. Hunter DJ, Rimm EB, Sacks FM, et al. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol 1992;135:418–27.
31. Enig MG, Atal S, Keeney M, Sampugna J. Isomeric *trans* fatty acids in the US diet. J Am Coll Nutr 1990;9:471–86.
32. Takatori T, Phillips FC, Shimasaki H, Privett OS. Effects of dietary saturated and *trans* fatty acids on tissue lipid composition and serum LCAT activity in the rat. Lipids 1976;85:272–80.
33. Moore CE. Alfin-Slater RB, Aftergood L. Effect of *trans* fatty acids on serum lecithin: cholesterol acyltransferase in rats. J Nutr 1980;110: 2284–90.
34. Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. Harper's biochemistry. 27th ed. Norwalk, CT: Appleton and Lange, 1990.
35. Emken EA. Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Annu Rev Nutr 1984;4:339–76.
36. Shekelle RB, Shryock AM, Paul O, et al. Diet, serum cholesterol, and death from coronary heart disease. The Western Electric Study. N Engl J Med 1981;304:65–70.
37. Arntzenius AC, Kromhout D, Barth JD, et al. Diet, lipoproteins, and the progression of coronary atherosclerosis. N Engl J Med 1985;312:805–11.
38. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of lipids, apolipoproteins and risks of myocardial infarction. N Engl J Med 1991;325:373–81.